



Prevalence of *Mycobacterium tuberculosis* Beijing genotype and its association with drug resistance in North India

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Abstract The global presence and rapid dissemination of Beijing genotype of *Mycobacterium tuberculosis*, makes it an important issue of public health. Its presence and association with multi-drug resistance has been shown in many settings. In present study we tried to find its prevalence and association with drug resistance in North India. One hundred and twenty four *M. tuberculosis* isolates were analyzed with spoligotyping, further drug susceptibility testing was done by 1% proportional method. Out of these, 11 (8.9%) *M. tuberculosis* isolates were identified as Beijing and 113 (91.1%) as non-Beijing genotypes. While looking at their drug susceptibility patterns, 6 (54.5%) & 22 (19.5%) were found to be multi drug resistant

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(MDR) among Beijing and non-Beijing isolates respectively. Our study concluded that the Beijing strains were not so common in north India and these strains do not fully associate with MDR.

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Introduction

Beijing family genotypes of *M. tuberculosis* (MTB) were first recognized in 1995 and are accounted for 86% of the tuberculosis isolates from Beijing, China, and a high proportion of isolates from Mongolia, South Korea, Hong Kong, Malaysia and Vietnam. In other regions like Finland and India, the presence of Beijing family genotype is relatively rare [1]. The *M. tuberculosis* belonging to Beijing family has increased ability to spread, cause the diseases and has higher association with drug resistance in comparison to non-Beijing MTB isolates [2]. In previous studies from Germany, Cuba, Estonia, Russia, and South Africa it was documented that the transmission of drug resistance have association with Beijing family genotype strains [3]. Strain W which is a highly drug resistant strain; reported from the United States also belongs to the Beijing family [3].

Outbreaks of MDR tuberculosis due to Beijing genotype have been reported in several parts of the world [4]. Researchers are concerned that the Beijing genotype may have a predilection for developing drug resistance and may be spreading worldwide, perhaps as a result of increased virulence [3,5]. Thus, a better understanding of the clinical and epidemiological relevance of *M. tuberculosis* Beijing/W lineage may allow the development of better strategies for TB control.

In the present study, we have attempted to assess the prevalence of *M. tuberculosis* Beijing genotype and its drug resistance pattern in North India.

Materials and methods

Strains

One hundred and twenty four randomly selected *M. tuberculosis* isolates from three different locations of North India were included, among these, 83

from eastern Uttar Pradesh (SA-1), 26 from Sawai Madhopur, Rajasthan (SA-2) and 15 isolates from Buxar, Bihar (SA-3). For the isolation of these MTB isolates, the specimen were collected during Oct. 2004-Dec. 2007 for SA-1 and for SA-2 and SA-3 the duration was Oct. 2004 to March, 2005. The specimens were processed by modified Petroff's method and inoculated on Lowenstein Jensen (LJ) media in duplicate. After further incubation the growth was observed and the MTB isolates were characterized using standard methods like; niacin test, growth on PNB containing media and catalase test [6] at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

DNA isolation

DNA was isolated as previously described [7]. In this method the DNA was extracted by phenol and chloroform. After extraction the DNA was precipitated by 70% ethanol and kept in TE (Tris EDTA buffer, pH 8.0).

Spoligotyping

Spoligotyping was performed on genomic DNA to detect the Beijing genotype (1–34 spacers absent) by using the standard method [8] with the help of commercially available kit (Isogen Biosciences, BV, Maarsse, The Netherlands). In brief the DR region was amplified by previously described sets of primers. After amplification the amplified DNA was hybridized on to the membrane which contains the corresponding probe for each spacer (1–43). The hybridization pattern was visualized with a chemiluminescence system, using Enhanced Chemiluminescent detection system (Amersham Biosciences, Buckinghamshire, United Kingdom). Proper controls (H37Rv, *M. bovis* BCG and Negative control) were used with each experiment. The pattern of each isolate was compared with the international spoligotyping database SpolDB4.0 [9].

Drug susceptibility test

Drug susceptibility testing of the *M. tuberculosis* isolates was performed by standard 1% proportion method on LJ-medium using *M. tuberculosis* strain H37Rv as sensitive control [10]. In this drug susceptibility method the isolate was called resistant if 1% or more proportion of colonies were growing on drug containing media, compared to drug free medium.

Results

Spoligotyping

Of 124 *M. tuberculosis* isolates for which spoligotyping was carried out, the majority of isolates belonged to shared type ST26; 22 (26.51%) in SA-1 [11]; 2 (7.69%) in SA-2 and 2 (13.3%) in SA-3. Out of these, 11 isolates belonged to Beijing genotype. Among these 11 isolates, 9 (10.8%) were from SA-1 [11] and 1 isolate each was found in SA-2 (3.8%) and SA-3 (6.6%). Amongst 11 isolates, 10 were represented by ST1 and 1 by ST1651 (Table 1).

Drug susceptibility test

All one hundred twenty four isolates (one hundred thirteen non-Beijing and eleven Beijing) were subjected for drug susceptibility testing. Among these,

6 (54.5%) Beijing strains of *M. tuberculosis*, were MDR whereas in non-Beijing isolates; 22 (19.5%) were MDR. Three (27.3%) and 85 (75.2%) isolates were sensitive to all drugs in case of Beijing and non-Beijing strains of *M. tuberculosis* respectively. Resistance to isoniazid ($n=7$, 63.6%) was much higher in Beijing strains than non-Beijing ($n=26$, 23.0%). The finding of isolates which were resistant to any drug was much lower ($n=28$, 24.2%) in non-Beijing than in Beijing isolates ($n=8$, 72.7%) (Table 2).

Discussion

Mycobacterium tuberculosis Beijing strains are prevalent in many parts of the world and often give rise to large institutional outbreaks. To the best of our knowledge this is the first initiative to assess the prevalence of Beijing genotype of tuberculosis and its association with drug resistance from three study areas. These study areas are the part of three major states of the India. In the earlier studies it was well documented about presence of Beijing genotype of MTB. The highest prevalence has been detected in Asia and Eurasia, while relatively low prevalence of Beijing strains has been reported from Western Europe [12,13].

In present study, by analysis with spoligotyping, we found the prevalence of *M. tuberculosis*

Table 1 Spoligotype family and subfamily distribution in three study areas analyzed with SpolDB4.

Family	SA-1		SA-2		SA-3	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
CAS1.D	25	30.1	6	23.1	2	13.3
CAS	—	—	1	3.8	—	—
CAS2	2	2.4	—	—	—	—
BEIJING	9	10.8	1	3.8	1	6.7
T1	8	9.6	1	3.8	1	6.7
T2-T3	1	1.2	—	—	—	—
T3	2	2.4	—	—	1	6.7
EAI3_IN	3	3.6	2	7.7	—	—
EAI1_SOM	2	2.4	—	—	—	—
EAI5	3	3.6	5	19.2	—	—
EAI 5/3	—	—	1	3.8	1	6.7
H1	2	2.4	—	—	—	—
H3	1	1.2	—	—	—	—
H4	4	4.8	—	—	—	—
MANU1	1	1.2	1	3.8	1	6.7
X3	—	—	1	3.8	—	—
U	1	1.2	—	—	—	—
Orphan	19	22.9	7	26.9	8	53.3
Total	83	100	26	100	15	100

Table 2 Drug resistance profile of *M. tuberculosis* isolates belonging to Beijing genotype and non-Beijing genotype.

S. no.	Profile	No. of isolates (n = 11)		No. of isolates (n = 113)	
		n	%	N	%
1	Sensitive to all drugs	3	27.3	85	75.2
2	Resistant to all drugs	3	27.3	12	10.6
3	MDR	6	54.5	22	19.5
4	Any drug resistance	8	72.7	28	24.8
5	Resistant to Isoniazid	7	63.6	26	23.0
6	Resistant to Ethambutol	5	45.5	19	16.8
7	Resistant to Streptomycin	6	54.5	15	13.3
8	Resistant to Rifampicin	6	54.5	23	20.3
9	Resistant to Isoniazid alone	0	0	2	1.8
10	Resistant to Ethambutol alone	1	9.1	5	3.5
11	Resistant to Streptomycin alone	0	0	1	0.8
12	Resistant to Rifampicin alone	0	0	0	0
13	Resistant to HES	3	27.3	10	8.8
14	Resistant to HER	4	36.4	13	11.5
15	Resistant to ESR	3	27.3	8	7.0
16	Resistant to HSR	3	27.3	13	11.5
17	Resistant to HE	4	36.4	13	11.5
18	Resistant to HS	6	54.5	12	10.6
19	Resistant to ES	3	27.3	9	8.2
20	Resistant to ER	4	36.4	14	12.4
21	Resistant to SR	5	45.5	9	8.2

Beijing strain was 8.9% (11/124), which is comparable with other Indian studies [14–17]. Two Indian studies published from New Delhi (8%) and Bombay (7.4%) showed similar prevalence of *M. tuberculosis* Beijing strain [14,15], whereas two other studies from same places have reported the prevalence to be slightly lower, i.e. 3.8% and 3% respectively [16,17]. Two studies from South Korea and Hong Kong reported the prevalence of Beijing strains to be 77% and 70% respectively [18,19]. Similarly studies from Yangon, Myanmar and Russia also reported a high prevalence of 31.9% and 42–68.1% respectively [20,21]. Another study from Malawi showed that 4.3% patients have Beijing genotype [22]. In our study the prevalence of Beijing genotype is low, so we can say that the Beijing genotype is not a dominant genotype in North India.

In our study we also analyzed the association of *M. tuberculosis* Beijing strain with drug resistance. The MDR cases were found in 6 (54.5%) isolates out of 11 *M. tuberculosis* Beijing strains, whereas a study from Bombay showed only 12.5% to be MDR [15], whereas the MDR were found 19.5% in cases of non-Beijing MTB. This finding suggests that the Beijing genotypes are associated with drug resistance at significantly higher percentage than non-Beijing family in this study. A study from China has reported a low percentage difference of MDR cases between

Beijing family (11.3%) and non-Beijing family (7.4%) [23]. In our study, the Beijing family has higher proportion in INH resistant (63.6%) in comparison to non-Beijing (23.0%). In the Chinese study the INH resistance was found quite similar to our study in case of non-Beijing isolates (18.4%) but much lower in case of Beijing isolates (21.7%) [23]. A study from Delhi reported that out of nine Beijing isolates, seven were resistant to EMB, SM, and INH, and one isolate was resistant to all four drugs tested [14]. A South African study reported that among the patients with Beijing strains, 50 (15%) from the urban setting and 63 (23%) from the rural setting had drug-resistant tuberculosis [4]. Among Beijing strains; in our study resistance to any drug was found in 72.7%, while a Korean study reported any drug resistance and MDR were 69.6% and 33.4% respectively [18]. While comparing with this; the non-Beijing genotype had very low percentage (24.8%) of isolates that were having any drug resistance. This finding also indicates that the rate of developing drug resistance is higher in Beijing genotype rather in non-Beijing genotype of MTB. Surprisingly a study from Madrid reported that all isolates were susceptible to anti-TB drugs, except 1 streptomycin-resistant strain among the Beijing strains [24]. This finding is not in a concordance with our study.

Conclusion

Since this study was focused on set of samples that were probably less than representative, it is difficult to draw any concrete conclusions on the relationship between Beijing family genotype strains and its association with the resistance to all first-line antituberculosis drugs. But by this finding we can conclude that the Beijing genotype of *M tuberculosis* is not always associated with MDR but it is much higher than non-Beijing MTB.

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Competing interests

None declared.

Ethical approval

Not required.

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